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The role of oxysterols in the regulation of cholesterol biosynthesis

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Most mammalian cells are able to satisfy their requirements for cholesterol from either external (lipoprotein) or internal (biosynthetic) sources. Although the rate of cellular cholesterol biosynthesis appears to bear an inverse relationship to the availability of external lipoprotein cholesterol, the exact nature of the regulatory molecule(s) remains to be unequivocally established. Several oxygenated derivatives of cholesterol (oxysterols) are powerful inhibitors of cholesterol biosynthesis, an effect which appears to be due primarily, but not exclusively, to a decrease in the activity of HMG-CoA* reductase. One of the aims of the present paper is to examine the relationship between oxysterol structure and inhibitory potency. Various hypotheses have been proposed to explain the means by which these compounds exert their effects at the molecular level and these will also be discussed. However, the main objective of this paper is to review the evidence for the natural involvement of a component of this type in the normal regulatory sequence of events culminating in the suppression of cellular cholesterol biosynthesis.

Relationship between oxysterol structure and inhibitory activity

Purified, non-lipoprotein cholesterol is a very poor inhibitor of cholesterol synthesis and of HMG-CoA reductase when added to the culture medium of several different types of cell *in vitro* (Bell *et al.*, 1976; Kandutsch *et al.*, 1978; Krieger *et al.*, 1978; Peng *et al.*, 1979; Drevon *et al.*, 1980). It is probable that the inhibitory effects of cholesterol described in previous reports (*e.g.* Rothblat & Buchko, 1971) resulted from steroidal contaminants of cholesterol rather than from cholesterol itself (Kandutsch *et al.*, 1978). These inhibitors have been identified as oxygenated cholesterol derivatives such as 7-oxocholesterol, 7α- and 7β-hydroxycholesterol and 25-hydroxycholesterol, all of which arise spontaneously from cholesterol by air oxidation. The ready susceptibility of cholesterol to such free-radical-induced air oxidation is such that it is difficult, during longer incubation periods, to completely exclude their non enzymic formation even from highly purified cholesterol added initially.

* Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; LDL, low-density lipoprotein.

Table 1. *Effectiveness of oxysterol types in the inhibition of HMG-CoA reductase in L-cells (all sterols contain an oxygen function at C-3)*

Position of additional oxygen function	Range of activity [concentration required for 50% inhibition (μM)]	Number of steroids tested	Most powerful inhibitor
4	>15	1	5α-Cholestane-3β,4β-diol
6	1.5–9.0	4	3β-Hydroxy-5α-cholestan-6-one
7	2.0–10.0	4	3β-Hydroxy-5α-cholestan-7-one
9	1.0–3.0	2	5α-Lanostane-3β,9α-diol
15	0.3–8.8	20	Several
32	0.5–3.3	10	14α-Hydroxymethylcholest-8-en-3β-ol
20–25	0.05–3.5	5	25-Hydroxycholesterol

The biological activity of these oxysterols stimulated a search for more effective derivatives and to-date well over one hundred oxysterols have been synthesized and tested for biological activity, mainly in the laboratories of Kandutsch and Schroepfer. In general, purified monohydroxy- and mono-oxo-derivatives of 5α-cholestane and 5α-lanostane are relatively ineffective in decreasing HMG-CoA reductase activity (Kandutsch & Chen, 1973, 1974). However, introduction of a second oxygen function into the molecule in addition to that at C-3 usually gives rise to a large increase in inhibitory activity. The position of the second oxygen function is of some importance in determining the inhibitory power of the derivative and Table 1 shows the activities of oxysterols classified according to substituent position. Although each sterol type shows a rather wide range of activity, selection of the most potent sterol in each group shows that, in general, the greater the molecular distance between C-3 and the second oxygen group, the greater the inhibitory activity. An intact (i.e. an iso-octyl) side-chain is also required for full activity, a gradual decrease in the length of the side-chain resulting in a gradual diminution in biological effectiveness (Kandutsch & Chen, 1974). Within some groups of steroids there appears to be an inverse relationship between inhibitory effectiveness and the extent to which the oxygen function is sterically hindered (Gibbons, 1983). For example, as the 15 hydroxy group becomes increasingly shielded by the presence of more bulky substituents at C-14, so the potency of the resultant steroid decreases (Schroepfer *et al.*, 1979). In addition, introduction of axial hydroxy groups at the 3α-, 6β-, 7α- or 15β-positions produces steroids which are less inhibitory than those in which each hydroxy group is in the corresponding, less hindered equatorial conformation. Oxygen functions in the conformationally flexible positions such as those in Ring D and in the side-chain also appear to produce more inhibitory steroids and it has previously been suggested that the biological potency of steroid hormones is dependent upon conformationally flexible functional groups which permit effective hydrogen bonding or hydrophilic interactions with receptor molecules (Romers *et al.*, 1974; Duax *et al.*, 1980).

Molecular mechanism of oxysterol action

The sequence of events initiated by the entry of an oxysterol into (or formation within) the cell and culminating, amongst other effects, in the suppression of HMG-CoA reductase, is largely unknown. However, there is evidence that, as for some steroid hormones, there is a specific cytosolic receptor protein for oxysterols (Kandutsch & Shown, 1981). In general, the affinity with which this protein binds a particular oxysterol is directly related to the effectiveness of the steroid in inhibiting HMG-CoA reductase.

Much of the effort in this field has been confined to attempts to determine whether oxysterols decrease HMG-CoA reductase activity by decreasing the steady-state concentration of the enzyme (quantitative effects) or by decreasing the efficiency of pre-existing enzyme (qualitative effects). There is currently no general agreement as to which of these mechanisms is correct. However, any proposal involving modification of pre-existing enzyme would have to explain why the oxysterol-mediated suppression of enzyme activity requires not only the intact, integrated cell structure (Kandutsch & Chen, 1975; Erickson *et al.*, 1978; Cavenee *et al.*, 1981) but also continuing protein

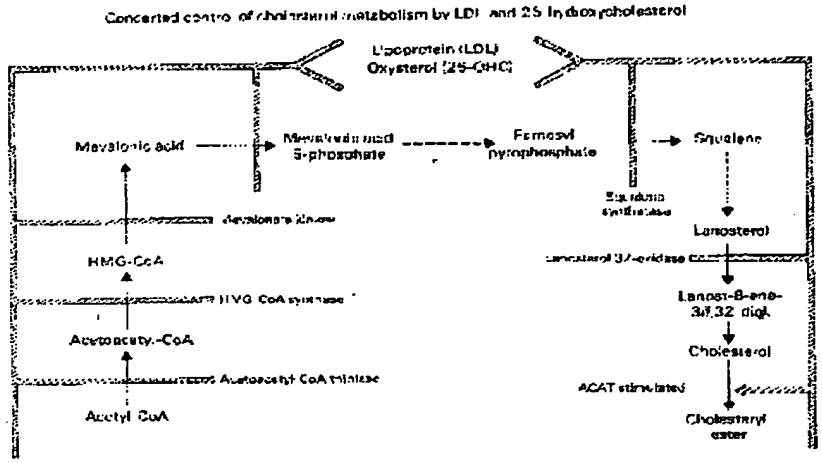

Fig. 1. *Identical effects of LDL and 25 hydroxycholesterol (25-OHC) on cholesterol metabolism*

Abbreviation used: ACAT, acyl-CoA : cholesterol acyltransferase.

synthesis (Chang *et al.*, 1981). There is a substantial body of evidence (see below) which suggests that the characteristics of suppression of cholesterol synthesis by LDL are identical with those of inhibition by oxysterols (e.g. 25 hydroxycholesterol). In view of this, since LDL appears to suppress HMG-CoA reductase activity in a compactin-resistant strain of Chinese hamster-ovary cells by preventing the synthesis of reductase-mRNA (Chin *et al.*, 1982), it is reasonable to believe that 25-hydroxycholesterol, which also suppresses HMG-CoA reductase activity in these cells, does so by an identical mechanism. If this is the case, then the primary effect of the oxysterol-induced inhibition of reductase activity results from a decreased rate of synthesis of enzyme protein. In addition, somewhat circumstantial evidence for this view derives from the apparent similarity between the modes of action of the glucocorticoids, which are known to inhibit enzyme synthesis by suppression of the relevant mRNA, and the oxysterols, insofar as the activity of both classes appears to be mediated by binding to receptor proteins (Kandutsch & Thompson, 1980).

Are oxysterols natural regulators of cholesterol biosynthesis?

It is well known that an increase in the supply of cellular cholesterol derived either from external lipoprotein (e.g. LDL) or from mevalonic acid, which is readily converted, enzymically, into cholesterol, is associated with a decreased activity of HMG-CoA reductase. However, there is no convincing evidence that, in either case, cholesterol itself is the inhibitory molecule in the intact cell. In fact, as mentioned above, pure, non-lipoprotein cholesterol, unlike LDL, has, over the shorter term, relatively little effect on reductase activity in several types of cultured cell *in vitro*. This, of course, may result from a different subcellular localization of cholesterol delivered to the cell by different means. However, it is noteworthy that although the effects of lipoproteins cannot be reproduced by cholesterol, the characteristics of the lipoprotein(LDL)-mediated suppression of cholesterol synthesis are identical with those of suppression by oxysterols, particularly 25-hydroxycholesterol. Thus, apart from reductase, which appears to be the most sensitive, LDL added to intact cells suppresses a wide range of enzymes involved in cholesterol synthesis (Fig. 1) (Chang & Limanek, 1980; Gibbons, 1983). In addition, LDL suppresses LDL-receptor activity and stimulates acyl-CoA : cholesterol acyltransferase. 25 Hydroxycholesterol faithfully mimics LDL in reproducing each and every one of these effects in various cells. Thus the fingerprint pattern of inhibition of cholesterol synthesis by LDL is matched exactly by that of oxysterols. Further evidence that the effects of external lipoproteins are mediated by an oxygenated sterol derivative has been obtained by the specific selection of cells which are resistant to the suppression of HMG-CoA reductase by oxysterols (Chen *et al.*, 1979; Sinensky *et al.*, 1979; Chang & Limanek, 1980). All of these cell lines are concomitantly resistant to suppression by serum lipoproteins, strongly suggesting that the latter either contain an inhibitory oxysterol or give rise to one after uptake by the cell.

If an oxysterol is indeed responsible for mediating the effects of LDL on cholesterol metabolism, then its origin is obscure. One possibility is that oxysterols arise from spontaneous, non-enzymic oxidation of LDL-cholesterol or from dietary cholesterol (Schroepfer, 1981). However, it is not certain whether oxysterols derived from this source are primarily responsible for the biological effects observed. Another alternative is that after endocytosis, LDL gives rise to the formation of an oxysterol derivative either by a controlled, enzymic oxidation of lipoprotein cholesterol or by interference with the pathway of endogenous cholesterol metabolism in such a way that a regulatory steroid accumulates (see below).

As regards the suppression of HMG-CoA reductase activity during periods of accelerated cholesterol synthesis from exogenous mevalonolactone (Edwards *et al.*, 1977; Gould, 1977), here again there is no unequivocal evidence that biosynthetic cholesterol is responsible for the effects observed. It is important to bear in mind that under these circumstances, the rate of production of all post-mevalonate cholesterol precursors is probably enhanced. Two such precursors are 5α lanost-8-ene-3β,32-diol and 3β-hydroxy-5α-lanost-8-en-32-al (Akhtar *et al.*, 1978; Gibbons *et al.*, 1979), which arise during the 14α-demethylation of lanosterol. Cytochrome *P*-450 is required for the initial oxidation resulting in 5α-lanost-8-ene-3β,32-diol (or the Δ^{24} derivative) and the inhibition by some oxysterols of the further metabolism of lanosterol is most probably due to competitive inhibition of cytochrome *P*-450 by the oxysterol (Gibbons *et al.*, 1979; Ortiz de Montellano *et al.*, 1979). Both the above oxysterol intermediates of lanosterol 14α-demethylation are effective inhibitors of HMG-CoA reductase *in vitro* (Gibbons *et al.*, 1980). Thus caution should be exercised in inferring a direct causal relationship between biosynthetic

cholesterol production and HMG CoA reductase activity under conditions during which the rate of synthesis of other, inhibitory, natural precursors of cholesterol also increases. In addition to cholesterol precursors, regulatory oxysterols also arise naturally as a result of cholesterol catabolism in certain types of cell. For example, 20α hydroxycholesterol and 7α-hydroxycholesterol arise in certain endocrine organs and in liver respectively, during steroid hormone and bile acid formation (Kandutsch *et al.*, 1978).

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Regulation of acyl-coA:cholesterol acyltransferase

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The formation of cholesteryl esters in cells was first described in the 1950s (Mukherjee *et al.*, 1958). However, the potential importance of this process and the enzyme catalysing it, ACAT,* was particularly highlighted by the proposal of the low-density-lipoprotein-receptor hypothesis in 1976 (Brown & Goldstein, 1976). ACAT activity has been found in a wide range of tissues and cell types [see Spector *et al.* (1979) for a review of the literature up to 1979]. More recently activity, previously thought to be absent, has been convincingly demonstrated in human liver (Erickson & Cooper, 1980; Balasubramaniam *et al.*, 1981). Substantial activity has also been reported in the intestine (Helgerud *et al.*, 1981; Field *et al.*, 1982; Field & Salome, 1982; Norum *et al.*, 1983; B. F. Stange, K. E. Suckling & J. M. Dietschy, unpublished work). It had been the general view that the pancreatic cholesterol esterase was responsible for cholesteryl ester formation in intestinal epithelium (Gallo & Treadwell, 1963) but the more recent work makes it clear that the ACAT activity can account for the entire cholesterol-esterifying activity of these cells.

One difficulty in comparing work on ACAT from different sources is the various methods of assay used (Spector *et al.*, 1979). Much of the earlier work used an assay which was based on the incorporation of labelled exogenous cholesterol into cholesteryl esters. Because the microsomal fractions in which ACAT is found contain significant amounts of endogenous cholesterol, which is distributed into several pools of unknown size and function, it is not possible to determine an absolute rate of cholesteryl ester formation when sterol is added as a tracer. Most recent work has used a labelled fatty acid (for work in intact cells) or, better, labelled fatty acyl-CoA for work on cell-free systems. This latter method does allow an estimate of the rate of cholesteryl ester formation to be made *in vitro*, but because of differences in assay procedure the absolute values reported by different laboratories are not always comparable. The reported methods vary in the fatty acyl-CoA used, the presence of fat-free bovine serum albumin and the use of detergents or additional unlabelled cholesterol. Dietary factors, which may vary from laboratory to laboratory, are also important. Thus published values for ACAT activity in rat liver microsomal fractions range from 20 to 400 pmol/min per mg of protein. There can also be a large variation between groups of similar animals kept in identical conditions (Erickson *et al.*, 1980). It is therefore essential to relate experimental values to the appropriate internal controls.

Much attention has focused on the regulation of ACAT activity, since, according to the low-density-lipoprotein receptor mechanism, ACAT is activated on uptake of low density lipoproteins and HMG-CoA reductase is inhibited. Table 1 summarizes some of the evidence for regulation of ACAT activity from manipulations *in vivo*. It appears that, although under many circumstances ACAT and HMG CoA reductase activities respond inversely to dietary manipulations, there are a number of occasions (for example, cholestyramine administration and a number of treatments on rat intestinal cells) where this is not the case. It is likely that uptake of exogenous cholesterol into a cell by any of the possible mechanisms is a major factor in regulating ACAT activity (K. E. Suckling & J. M. Dietschy, unpublished work).

Studies *in vitro* also lead to the view that substrate supply is important in determining the measured ACAT activity. Addition of exogenous cholesterol to microsomal fractions from liposomes or in detergents or organic solvents all lead to

* Abbreviations: ACAT, acyl CoA:cholesterol acyltransferase (EC 2.3.1.26); HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA reductase.